Evaluation of Heavy Metal Levels in the Serum of Breast Cancer Patients in Port Harcourt, Nigeria

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ABSTRACT

Introduction: Breast cancer is a form of cancer that originates from the breast tissue. It's a disease where cells in the breast grow swiftly and advance beyond regulation. Heavy metals such as copper (Cu), chromium (Cr), cadmium (Cd), zinc (Zn), lead (Pb) and nickel (Ni) were analysed in the serum of breast cancer patients by atomic absorption spectroscopy (AAS), to determine the possible link between human environmental exposure to these metals and increased risk of breast cancer in Port Harcourt, Nigeria.

Material and methods: 150 women were implicated for this study. 100, which were serologically and histologically confirmed as positive for breast cancer, served as test patients and 50 women with no known individual or family history of breast or any other type of cancer served as control.

Results: The metals analysed were all found to be elevated and statistically significant ($p<0.05$) in the breast cancer patients when compared to the control patients. The elevated levels of these metals could either have contributed to the cause of the breast cancer by disrupting endocrine function through the creation of reactive oxygen species (ROS) or a consequence of the breast cancer.

Conclusion: The results obtained in this study points to the existing proof obtained in other countries that sustained exposure to these metals, especially in an industrial vibrant environment, pose great risk to breast cancer and/or any other type of cancer.

Keywords: Heavy Metals, Breast Cancer, Serum, Atomic Absorption Spectrometry.

INTRODUCTION

There are different types of breast cancer depending on which cells of the breast become cancerous.[1] Signs may include a breast lump, breast shape change, skin dimpling, fluid dripping from the nipple, and red scaly patch of skin. Others are bone pain, swollen lymph nodes, shortness of breath and yellow skin.[2] The breast is made up of three main parts: lobules (glands that produce milk), ducts (tubes that carry milk to the nipple) and connective tissue (fibrous and fatty tissue that surrounds and holds everything together). There are different kinds of breast cancer, which may be a single type, a combination or a mixture of invasive and non-invasive type.[1] Ductal, lobular and inflammatory breast cancers describe the different types based on the origin.

The main causes of breast cancer are many and most times still unknown. Knowledge of breast cancer biology could help and guide the understanding to how and why. Many different factors are thought to play a role. Hormonal influences, endocrine and reproductive factors, toxic environmental compounds as well as oxidative stress and lipid peroxidation have been suggested to play a role in the carcinogenesis of breast cancer.[3]

Over the years, enhanced knowledge of the significance of heavy metals as regards human health has led to approach in technology that eased their analysis in body fluids and tissues[4] and the increased attention in the knowledge of the main role heavy metals are involved in different disease states has led to several studies in understanding the probable link. However, heavy metals are a small part of the biology of the human systems responsible for essential biochemical processes in very reduced concentrations. Also, several diseases whose cause was not known before now have been linked to variation in the levels of these metals, either in excess or deficiency. Due to the basic importance of heavy metals in several functions of the human body, it is logical to hypothesize that their variation in the body may enhance some biochemical processes such as creation of reactive oxygen specie (ROS), which could lead to diseases such as cancer, stroke, diabetes etc.[5]

Breast cancer has overtaking cervical cancer as the most diagnosed cancer in women in most part of the world[6], Port Harcourt, Nigeria included. Even when the incidence is lower in developing countries, the burden and mortality rate of the disease is far greater as a result of poverty, education, poor health facilities, religious as well as cultural practices leading to poor and late diagnosis. The various known risk factors for breast cancer which include female sex, age, hormones, early puberty (menarche), late menopause, null parity, having the first child at a late age, BRCA genes, family history of breast or any cancer[7] have been established to not really clarify the high incidence and differences in geographical location linked to the disease[8], as research has shown that half the women with breast cancer do not have the already known and proven risk factors besides the female sex and age.[9] Breast cancer as a disease has been known to be hormone (oestrogen and progesterone) dependent but heavy metals as environmental factors act as endocrine disrupters

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at cellular or subcellular levels via conflicting mechanisms, one of such could be the relationship between heavy metals and hormones that control the metabolic process of the human body.[10]

According to World Health Organization,[11] breast cancer remains the highest solid tumour diagnosed mostly in females and genesis of cancer mortality in women. There have been approximately 1.7 million new cases (25% of all cancer in women), 0.5 million-cancer deaths, and 15% of all cancer death in women recent years. Incidence rates are considered high in countries getting to the high level of human capital development with an increased 2-fold distinction between countries grouped into low to various high levels of development. There is an increased occurrence annually in nations with diverse industries and urban way of living with up to 70 – 90 new cases per 100,000 women as industrialization increases incidence and a high death rate.

The uniqueness of the breast tissue is in part due to its extensive hormonal influence and extraordinary changes during life. Each hormonal metabolism and range is affected by chemicals used readily, and by factors in the environmental, which are able to distort endocrine function and alter the function hormones.[12] A little more than half of the women with known breast cancer in the USA are not linked to known breast cancer risk factors other than age and the female sex[9] and research scientists indicate that toxic chemicals could aid the enhance of developing breast cancer. Research scientists reiterate that the weight of cancer triggered by environmental factors has been misconstrued greatly as people in developing as well as the developed nations are constantly battered with different combinations of dangerous exposures before they are born.[12] Several women with the known risk factors may not get breast cancer justifying the importance of identification of possible risks for breast cancer other than the established ones. The interest is now in environmental pollutants, possible endocrine disruptors that could be part of the unexplained breast cancer.[13,14] These compounds enter the body through air, food, water and cosmetics or through dermal exposure, through the plasma membrane to the cell in order to cause toxicity[15-17]. In Nigeria, despite the concern raised by clinicians on the upsurge of breast cancer among Nigerian women, literatures on the relationship of breast cancer to established risk factors such as heavy metals, which are described, as endocrine disruptors are rare. Therefore this present study was designed to evaluate the heavy metal levels in the serum of breast cancer patients in Port Harcourt, Nigeria.

MATERIAL AND METHODS

The study population consists of 150 patients, 100 patients were serologically and histologically confirmed as positive breast cancer patients of either ductal carcinoma, ductal carcinoma in situ and/or lobular carcinoma. These females presented at the Surgery Clinics of University of Port Harcourt Teaching Hospital, Braithwaite Memorial Specialist Hospital and Meridrien Hospital, Port Harcourt. 50 patients, who served as control, were individuals with no known individual or family history of either breast or any other type of cancer excluded by thorough physical examination.

Informed consent was obtained from each patient. Ethical approval was also obtained from the Ethics Committees of the various institutions before the commencement of the study. The patients were all recruited and implicated at the pre-surgical stage at the treatment centres.

Examination of the patients

A detailed history of all the subjects was recorded. This includes age of menarche, parity, duration of breastfeeding, family history if any, of breast cancer or any other type of cancer, place and duration of residence, occupation, use of tobacco as well as alcohol among other things. Detailed physical and clinical examination was performed on both breasts. The physical characteristics of lump on the breast and surrounding area, nipple examination, lymph node involvement and any sites of bone pain were properly examined. A serologically and histologically clinical diagnosis of breast carcinoma alongside the clinical stage was established.

Collection of Samples

Samples were collected from the patients at the treatment centres. Blood samples were collected in plain vacutainer sample bottles.

Experimental Design

This case controlled study comprised of two study groups, A and B. Group A consisted of serologically and histologically confirmed breast cancer patients while group B consisted of non cancerous patients with no risk, non proliferation benign breast diseases. Group B served as control.

Sample treatment

Blood samples were collected and preserved in the laboratory refrigerator before analysis. Serum was separated from whole blood and kept frozen in the refrigerator at a temperature of -2 to -8^{0}C until ready to use.

Analysis of heavy metals

Serum copper, zinc, chromium, lead, nickel and cadmium were determined using a solar thermo-elemental flame atomic absorption spectrometer (FAAS) (Model S4-71096) American standard. The serum samples were first treated by digestion with nitric and hydrochloric acids. Samples for elemental assays of different matrices, either organic or inorganic needs partial or absolute dissolution of the samples before assays with the instrument. Preparation of sample allows for the separation and /or the pre-concentration of analytes. The digested samples were assayed for heavy metals with FAAS with appropriate wavelengths and hollow cathode lamps.

STATISTICAL ANALYSIS

Stata Statistical Data Analysis version 12 was used. 2-group Hoteling's T-squared multivariate test was used to test for significance. Results are reported as means and standard deviation. Variation in means was set at < 0.05.

RESULTS

Table 1 shows the means ± SD of serum heavy metals in test and control female subjects in the population studied. The table shows that there is substantial significant ($p<0.05$) elevations in the levels of the heavy metals in the serum of the test subjects when compared with the levels in the control subjects. This is also shown in figures 1,2.

DISCUSSION

In this study, some heavy metal levels was investigated in normal and breast cancer patients' serum to determine the likely link between exposure to these metals and possible risk of breast cancer initiation among women in Port Harcourt, Nigeria and its surrounding environs. This is because there is suddenly an increased incidence in the number of breast cancer cases among women in this region. There is also high concentration of multinational industries in the region, which emit industrial effluents, heavy metals included, which are harmful to human health over a sustained period of time. These metals are suspected human cancer causing agents[15].

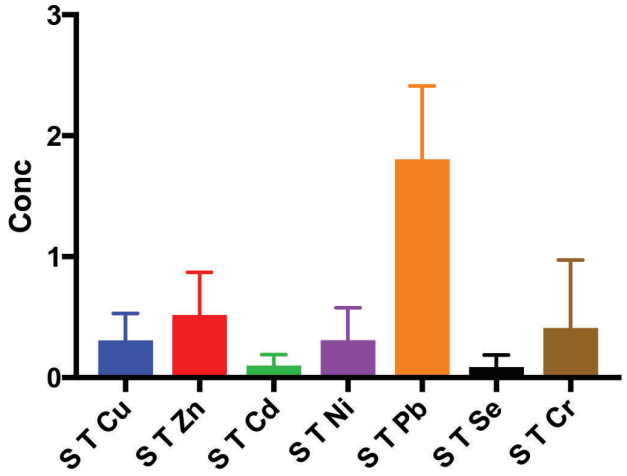

Figure-1: Serum heavy metals test subjects

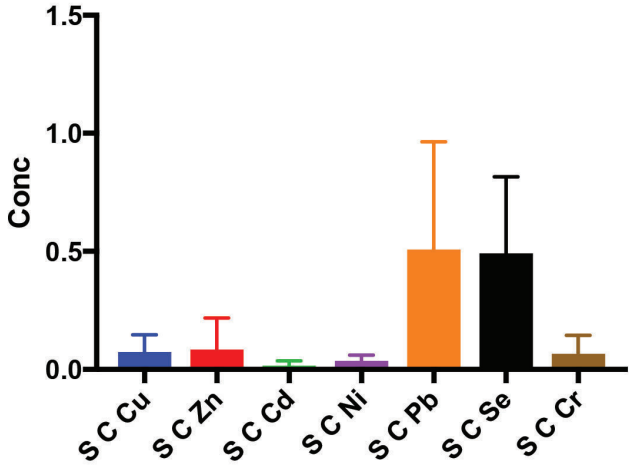

Figure-2: Serum heavy metals control subjects

The heavy metals Cu, Zn, Cd, Ni, Pb and Cr were identified and their concentrations in serum estimated. There is a significantly ($p<0.05$) increased level of all heavy metals assayed in the serum samples of women with breast cancer than in those without (table 1). Heavy metals have been reported to be involved in disrupting endocrine functions by creating reactive oxygen species (ROS). Studies have shown that these metals in sustained elevated states in the body acts as facilitators in the oxidative evaluation of biological macromolecules and elicit ROS, giving off free radicals as a consequence. Build up of ROS could affect epigenetic factors resulting to mutations and diseases, cancer included[16]. Though the observed increased incidence of breast cancer in the population studied could not conclusively attributed to the levels of the heavy metals, the result of this study suggests that these metals could play some roles in the initiation of breast cancer in women in this population.

Copper levels are significantly high ($p<0.000$) in the serum of breast cancer patients when compared to the non-breast cancer patients. According to Blockhuys[17] et al, serum levels of copper were reported to be increased in malignant breast cancer patients than in control patients and highest in the most advanced breast cancers. The increased level of Cu in the breast cancer patients is believed to aid breast carcinogenesis via angiogenesis and oxidative DNA damage[18].

Zinc level was also significantly ($p<0.000$) raised in the serum of breast cancer patients than in the control. Several authors[18], have reported that human breast cancer tissues contain raised levels of zinc compared to normal breast tissues used as control. Thus, the significant ($p<0.05$) increased concentration of zinc in the serum of the breast cancer patients obtained in this study agrees with the result of the above authors. Zinc level is directly linked to the function of the immune system and any modification in the level of zinc could cause defective immune function, elevating the likelihood of varying immune related and endocrine diseases, cancer included[19-21].

Significant ($p<0.05$) level of cadmium was also seen in the serum of the test patients when compared to the control. Females are believed to have increased Cd levels than males possibly due to lower iron stores that could aid Cd absorption[22,23], making the comparable environmental cadmium exposure more likely to affect females than males hence its role in breast cancer[24] (Olson et al., 2002). Schwerdtle[24] et al., in their in vitro study showed that both

Parameters	Cu (µg/l)	Zn (mg/l)	Cd (µg/l)	Ni (µg/l)	Pb (µg/l)	Se (µg/l)	Cr (µg/l)
Test N=100	0.309±0.22	0.519±0.35	0.100±0.09	0.311±0.266	1.807±0.60	0.089±0.10	0.411±0.56
Control (n=50)	0.074±0.07	0.085±0.13	0.010±0.03	0.037±0.02	0.508±0.46	0.492±0.32	0.067±0.07
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Remarks	S	S	S	S	S	S	S

Table-1: Mean + SD of serum heavy metals for test and control subjects

water soluble and particulate cadmium disturbs nucleotide excision repair of large DNA adducts and UVC-elicited DNA photo lesions, aiding the hypothesis that Cd acts as an indirect genotoxic agent. The carcinogenic outcomes of Cd could also be settled in part by its potential to obstruct with the function of p53, a key controller of several components of DNA damage elicited protection process[26] and by its potential to replace zinc in proteins important to cell integrity such as XPA, an enzyme critical to nucleotide excision repair[27]. In addition to breast and other cancers, there seems to be enough proof to show that Cd aids immensely in various regular diseases such as osteoporosis, stroke, heart failure and renal tubular damage[27]. The high level of breast cancer prevalent in the population studied, therefore, implicates cadmium as an aetiological agent.

Lead (Pb) also showed significant ($p<0.05$) higher values in the breast cancer patients than those of the controls. Lead, known to be toxic to various organs and tissues, impedes different body processes and the growth of the neuron system hence dangerous to children and if untreated could lead to possible life long cerebral behavioural disease[29]. The potential of lead to act as a potent oestrogen indicate that it could be a vital class of endocrine disruptors[30]. Environmental lead intoxication is important in the generation of ROS, which cause oxidative damage, i.e. the direct participation of lead in free radical reactions leading to elevated risk of breast cancers or tumours. The mean ± standard deviation of lead in serum of breast cancer patients and control subjects were 1.807± 0.605 and 0.508 ± 0.457 respectively indicating a clearly higher level than other metals measured. The inhibitory effect of inorganic and organic lead compounds on δ-aminolevulinic acid dehydrogenase (δ-ALAD) accounts for a build up of -aminolevulinic acid (d-ALA), which easily oxidizes to produce reactive oxygen specie (ROS) resulting in oxidative DNA damage, and indicating the feasible mechanistic or procedural basis of lead-elicited carcinogenesis[31]. Lead has an unfavourable effect on iron deficiency, which aids the absorption of lead and studies suggest that more lead is absorbed in the presence of low calcium than in high calcium concentration. The level of lead in the samples of malignant patients agrees with related studies. IARC[15], (1980), reported that lead is a known carcinogen in environmental studies, predicting lead as a likely activator of human cancer. Sources of environmental lead are leaded gasoline, pipes for water supply, lead based paints, untreated industrial wastes, ceramics, cosmetics.

Significantly ($p<0.05$) raised level of Nickel in the test patients when compared to control subjects was also observed in the study. This is in line with the study of Ionescu[30] et al., (2008). They observed very high level of nickel build up in 20 breast cancer tissue biopsies compared to controls. Nickel has been known to impede the repair of destroyed DNA. The carcinogenicity of nickel compounds are believed to be due to oxidative stress, DNA damage, epigenetic outcomes and the control of gene expression by activating certain transcription factors.

In this study, significantly ($p<0.05$) increased values of chromium were seen in the serum of breast cancer patients compared to the control. Chromium's mutagenic toxicity is mainly linked to the adverse effects of Cr (VI) compounds. The difference in adverse effects between Cr (111) and Cr (VI) is in part due to the way in which Cr (VI) is taken up by the cells. Cr (VI) is partly reduced to Cr (III) as it enters the cell. This produces genotoxic effects. Cr (VI) is able to cause unusual phenotypes as a result of ROS formation and various DNA lesions when in increased concentration. Environmental subjection of chromium is vital as Cr compounds pose a risk for endocrine related disease such as breast cancer at environmentally relevant doses. Chromium carcinogenesis could be via the repression of p53, the cancer cells suppressor protein. Deactivation of this protein through modifications is linked to changes in the process of p53 reliant cell arrest as against the role of repairing the destroyed cells and to various human cancers as the protein is involved in diverse biological procedure such as control of genes required in the cell cycle, impede of cell growth following DNA damage and apoptosis.

CONCLUSION

The recent surge in cases of breast cancer in Port Harcourt, Nigeria may not be attributable to the presence of known risk factors which are apparent in the population. The result of this study has shown that heavy metals might also contribute to the observed burden of the disease in the region. Therefore, routine analysis of the levels of these metals in women is recommended in our tertiary hospitals as an attempt to early detection of women who might be at risk of developing breast cancer. This effort shall immeasurably reduce the mortality rate of breast cancer amongst women in Nigeria.

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